FOSTERING COMMUNICATION AND COLLABORATION

The NIH Catalyst

A PUBLICATION FOR NIH INTRAMURAL SCIENTISTS

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The Research Behind the Plate

NIH's Vanity Fare

by Celia Hooper and Lee Mack

If you're willing to fork over an extra $50 every two years, the State of Maryland will let you choose any unique seven-character coinage for your license plate, provided it's not obscene or blatantly provocative. Most people use vanity plates to tell the world of their feelings for their car, their hobbies, or their spouses. But roam the NIH parking lots (which many of us do a lot these days) and it's science that catches your eye. *The NIH Catalyst* wanted to know about the research and researchers that drive these distinctive public displays of affection.

Vectors and Vehicles

VAXIN8 is on the Maryland Historic Vehicle license plate on the 1963 Landrover Series IIA 88 belonging to **David Kaslow**, head of NIAID's Malaria Vaccines Section in the Laboratory of Parasitic Diseases.

Since doing some fieldwork in Africa in 1992, Kaslow has been passionate about Landrovers (and actually owns two other nonworking Landrovers), as well as his quest to develop a malaria vaccine. And now that interest in that quest has increased in high places of late, Kaslow predicts an effective malaria vaccine is a decade away—give or take a few years.

The latest twist Kaslow is pursuing is a human vaccine to block transmission

continued on page 4

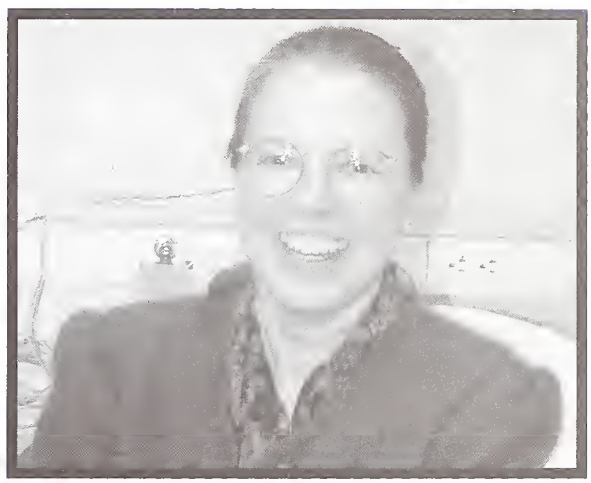
Celia Hooper

Nursing Research: New Directions for the 21st Century

by Fran Pollner

Science and clinical practice have been Annette Wysocki's two driving forces since her days as a student began to form the beginning of her life's chosen work.

But at first she perceived them as driving in different directions, science taking her exclusively to the laboratory, clinical practice to the bedside. It became a choice she had to make. She started out as a chemistry major at East Carolina University in Greenville, envisioning a career in research chemistry, and graduated with honors from that institution with a bachelor of science in nursing. She chose patient care.



Fran Pollner

Annette Wysocki

In the nearly two decades since Wysocki became a nurse and an active investigator, however, she's proved to herself and the world at large that doing science and being a nurse are not mutually exclusive.

In fact, Wysocki finds that the practice and the research enlarge one another in critical ways, to the benefit of nursing and all medical science and practice.

Wysocki is the person NINR director Pat Grady had been looking for from the time she arrived at NIH in April of 1995 as the second director of the then newest institute on the NIH campus: The National Center for Nursing Research. Created in 1986, it had only recently become the National Institute of Nursing Research, in accordance with provisions of the NIH Revitalization Act of 1993.

When she accepted the position of NINR director, Grady also assumed the designation as "acting scientific director" of the institute until she could recruit NINR's first permanent scientific di-

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Clinical Research Center Kickoff

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Training Scientists at NIH

Michael Gottesman

I was recently invited to an NIH Fellows' Committee town meeting to discuss my expectations regarding the training of scientists at NIH. NIH has approximately 2,500 postdoctoral fellows—designated as IRTAs, VFs, Guest Researchers, and Special Volunteers—in the intramural program, and there may be as many different points of view about what constitutes an outstanding training experience as there are fellows and mentors. I thought I would take this opportunity to offer mine.

While NIH has been schooling new waves of medical researchers for decades, and doing it well, improvement of mentoring and training here is a major goal of intramural leadership. My thoughts on the subject have sorted themselves into ten expectations of what postdocs should learn as a result of their NIH training. This learning process requires individual as well as institutional mentoring, in addition to active effort by the fellow. Most, but not all, of these objectives are also relevant to other training positions, such as the postbaccalaureate IRTA program and our pre-IRTA programs for graduate and medical students.

1. Doing Science: The primary goal of training here is to learn how to conduct innovative, high-quality science, including how to choose problems, choose model systems, develop logical hypotheses, design experiments, and see connections among different fields that allow a scientist to make quantum leaps in understanding a problem. Fellows choosing a laboratory with a proven track record or choosing to work with a compelling younger career investigator have already taken a step toward this goal. The NIH role in this realm is to ensure that only the highest quality research is done here and that opportunities for learning and critical discussion, such as through our lecture series and interest group meetings, abound.

2. Reading the Literature: A postdoc must learn to read the scientific literature critically. This requires access to library services, on-line information, journal clubs that dissect papers, as well as the chance to peer review "real" papers.

3. Communicating: A trainee must learn to communicate results, in writing and orally. A postdoc must, therefore, be given the opportunity to write papers and reviews and, in addition to journal club presentations, should expect to give a seminar at least once a year, and preferably more, on their ongoing work.

4. Conducting Ethical Science: It's essential that postdocs familiarize themselves with the "NIH Guidelines for the Conduct of Research" and have opportunities to discuss cases of scientific misconduct and the importance of integrity, honesty, and effective teamwork. Every postdoc should know where to turn if there is a problem, be it to the Women Scientist Advisors, the Office of Equal Opportunity, their scientific director, the NIH ombudsperson, or our Office of Intramural Research. Each postdoc should take the appropriate required coursework in radiation safety, laboratory safety, animal care and use, research on human subjects, and the ethics training required by their particular institute.

5. Forming Collaborations: Learning how to form and maintain collaborations requires guidance from supervisors and mentors. Good role models are important here. In addition, experience and discussions with colleagues, as well as information in the Guidelines, may help. A recent article in *The NIH Catalyst* discussing the finer points of collaboration can be found on page 3 of the July–August 1997 issue.

6. Choosing a Career Path: During the usual two to three years of postdoctoral experience at NIH, research activities may be highly supervised and directed toward the research goals of the particular laboratory, institute, or supervisor. During this period, a good mentor will provide frank career advice about whether a future as an independent researcher, a support scientist, or some other science-related field—such as science writing, technology transfer, or grants administration—is appropriate. If a postdoc stays beyond three years, means to foster graduated independence, or preparation for a support role or other scientific career, should be incorporated into the program, so that fellows are ready to move on to life after NIH.

7. Networking: Each fellow should have opportunities to meet scientists throughout NIH and at extramural sites. They should attend at least one scientific meeting a year to present their work, as posters or oral presentations; be introduced to other scientists; and make contacts about research materials and job opportunities. Mentors ought to encourage senior fellows in the lab to accept speaking invitations to make their work known to a wider scientific community. Fellows should also take advantage of similar opportunities on campus—the FARE award program, NIH Research Festival, departmental seminars, and interest group meetings and workshops.

8. Respecting Resources: Postdocs need to recognize that public funds that support their work carry a burden of responsible stewardship. Good science requires mastery of the basics of budgeting—of time, funds for research, and other scarce scientific resources, such as instrumentation, space, and personnel. Mentors ought include fellows in this budgeting process.

9. Mentoring: One goal of outstanding mentorship is to train students who themselves will be great mentors. Clearly, the process of mentorship is best taught by example, but fellows should also have the chance to supervise other students—and be guided and evaluated in that endeavor. More formal teaching experience, although not a primary objective of research training at NIH, is part of the mentorship process. My office is distributing a booklet on being a mentor entitled "Adviser, Teacher, Role Model, Friend," which was prepared under the auspices of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine and may be accessed at <http://www.nap.edu/readingroom/books/mentor/>. This booklet will be given to all principal investigators and to senior postdoctoral fellows leaving NIH.

10. Negotiating: Sometimes a fellow knows what resources are needed to succeed but lacks the negotiating or diplomatic skills to get them. Through example and advice, a mentor should teach a postdoc how to work through bureaucratic channels, how to convince others of the importance of their needs, and how to avoid antagonizing the very people who are pivotal in helping secure the desired items. The value of such skills should not be underestimated!

I hope this column generates serious discussions of the objectives of NIH training. I have asked the mentorship subcommittee of the NIH Committee on Scientific Conduct and Ethics to consider my list and to formalize an educational plan for our postdoctoral trainees. As always, I welcome your comments. ■

Interest Group Gazette

Therapeutic Oligonucleotides Interest Group

The second NIH symposium on Therapeutic Oligonucleotides will be held Friday, December 5, 1997, from 8:30 a.m. to 5:30 p.m. in Masur Auditorium (at the Clinical Center.) and will address "Targeting Transcription Factors and Signaling Pathways." For further information, contact:
Yoon Sang Cho-Chung
Head, Therapeutic Oligonucleotides Interest Group, NCI
Tel: (301) 496-4020
FAX: (301) 496-2443
Email: <yc12b@nih.gov>

Cornea Interest Group

They can't go on meeting like this, so after a year of meetings without benefit of official status, the Cornea Interest Group has formally organized as such. The group exchanges ideas on a variety of topics related to the ocular cornea, reviews papers in the field, and discusses current experimental findings, problems, and technical developments. Members are clinical and basic researchers from NIH and from local universities.
Meeting Time: first Monday of each month, 9:00-10:00 am
Meeting Place: Building 6, Room 412
Contact: Christina Sax
Phone: 402-4342
E-mail: <sax@helix.nih.gov>

Birth Defects and Teratology Interest Group

Contrary to information in the otherwise flawless Interinstitute Interest Group Directory pullout in the July-August Catalyst, the telephone number for contact person Dorothea de Zafra, of NIAAA, is 443-6516.

The interest group's web site is <http://www.nih.gov/sigs/birth-def/>.

Social Structure and Demographic Issues in Health Interest Group

The new e-mail address for contact person Laura Montgomery, NCHS/CDC, is <lem3@cdc.gov>.

In Vivo NMR Interest Group

The name of this new interest group was established at a September founding meeting, at which it was also decided that the group would be open to all intramural denizens with an interest in the science, technology, and in vivo application of magnetic resonance.

The group's mission is threefold: to encourage the use of MRI/MRS in biomedical applications, to promote interdisciplinary research and communication, and to disseminate information and provide consultative expertise.

The group intends to organize a seminar series and develop yellow pages of NIH groups involved in MRI research, a listserver with e-mail addresses of members, a calendar of events, and other vehicles for connecting people with similar research interests. They already have a web site: <http://www-mrips.cc.nih.gov/MRI>.

Meeting time: Alternate Wednesdays at 1:30 pm
For more info or to present your work during a Wednesday meeting, contact Jeff Duyn at <jhd@helix.nih.gov>. ■

Cytokine Interest Group Symposium

Thursday, December 11, 1997

Defining Cytokine Biology Through Knockout and Transgenic Mouse Models
co-chaired by Jeff Green and John Letterio
The Laboratory of Chemoprevention, NCI

8:50-9:00 Welcome/Opening Remarks: Jeff Green/John Letterio

Approaches to the Study of Cytokines Through Gene Manipulation
9:00-9:30 Reprogramming the Mouse by Directed Genome Manipulation: B. Sauer, NIDDK

9:30-10:00 Dominant Negative Approaches to Define Cytokine Function in Development and Oncogenesis: Glenn Merlino, NCI

10:00-10:30 Distinct Developmental Defects Associated with Disruption of FGF-Receptors: Chuxia Deng, NIDDK

10:30-11:00 coffee break (vendor displays in foyer outside auditorium)

11:00-12:00 Studies of TGF-b Superfamily Function Through Knockouts of Ligands, Receptors, and Interacting Proteins: Martin Matzuk, Baylor College of Medicine

12:00 lunch (catered)
Gene Knockout Models in the Study of Immune Funtion
1:00-1:25 Experimental Autoimmune Uveitis and Protective Oral Tolerance in Cytokine Knockout Mice: Luiz Rizzo, NEI

1:25-1:50 Single, Double, and Triple Gene Targeting at the TNF/LT Locus Using the Cre-loxP Recombination System: Sergei Nedospasov, NCI, Frederick

1:50-2:15 Cytokine Knockouts Define Mechanisms of Host Resistance to Intracellular Infection: George Yap, NIAID

2:15-2:40 TGF-b1$^{-/-}$ Mice Reveal a Link Between Expression of the cdk Inhibitor p21^{cip1} and Lymphocyte Survival: John Letterio, NCI

2:40-3:05 Evaluation of Lymphocyte Development in TCR Zeta Chain Null Mice: Paul Love, NICHD

3:05-3:30 The Role of Innate and Adaptive Immune Responses in Experimental Autoimmunity Defined Through Studies in Cytokine Knockouts: Ben Segal, NIAID

3:30 Concluding Remarks: John Letterio/Jeff Green

3:45–5:00 refreshments / discussion / poster session in the atrium

American Society for Cell Biology

December 13-17, 1997
Washington Convention Center

Just a subway ride away from NIH, the 37th annual meeting of ASCB features nine symposia and two dozen minisymposia on topics ranging from "Building the Brain" to "Nuclear Dynamics and Function."

For information, contact ASCB at 301-530-7153; fax: 301-530-7139; e-mail: <ascbinfo@ascb.faseb.org>.

Vanity Fare
continued from page 1

of the malaria sporozoan via its mosquito vector.

His plan is to create a vaccine that would generate antibodies to the parasite's gametes—the stage that is picked up by mosquitoes from infected individuals. When the mosquito takes a blood meal from a person who received this vaccine, the antibodies in the human blood would neutralize any parasite gametes the mosquito might be carrying, preventing zygote formation in the mosquito and any further transmission.

In addition, Kaslow's lab is working to develop a vaccine against the major surface proteins of the merozoites—the form of the parasite that lives in an infected person's blood and generates malarial fevers each time it bursts from blood cells. The ultimate goal, Kaslow says, would be to make a cocktail with both types of vaccine and VAXIN8 as many people as possible in malaria-rife regions of Africa.

Celia Hooper

David Kaslow
His quest to VAXIN8 against malaria, he hopes, will lead to antibodies at first bite.

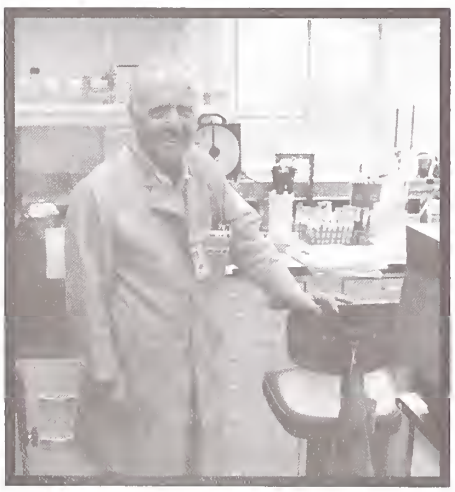
Rising Star

It seems only natural that **Enrico Cabib**, chief of NIDDK's Morphogenesis Section in the Laboratory of Biochemistry and Genetics, should bear the simple and unassuming declamation **YEAST** upon his license plate. Having worked intimately with this quiet member of the fungal kingdom for nearly 50 years, Cabib has developed a fondness for yeast that transcends beer brewing or bread baking. "Yeast is so small and defenseless," he says, "someone should vouch for it!"

Throughout a career he describes as taking him from being "a poor chemist to not-so-bad enzymologist to bad cell biologist," yeast has been Cabib's constant companion, first as a source of raw materials, such as sugar nucleotides, later for enzymes, and most recently as a tool for studying the molecular biology of morphogenesis.

Cabib's recent work has focused on the morphogenesis of the cell wall in budding yeast—a ripe target for developing new antifungal medications. Fungal diseases have always been a treatment challenge—especially in immunocompromised people—and have become more widespread in the past decade with the advent of AIDS.

Looking back a half-century before his own work started, Cabib notes that 1997 marks the 100th anniversary of modern biochemistry, a field that in his estimation owes its birth to his beloved YEAST, since it was extracts of yeast that Eduard Buchner used to accomplish his groundbreaking in vitro fermentation of glucose.



Lee Mack

Lee Mack

Enrico Cabib in his lab (left) and his movable yeast (above).

GABA-Fest

In 1989, looking for a focus for his lab's research, **Jeffery Barker**, chief of NINDS' Laboratory of Neurophysiology, settled on GABA, the neurotransmitter that regulates inhibitory and excitatory functions in the brain.

Trained as a physician, but propelled by an intellectual curiosity that, he says, surpassed his bedside manner, Barker came to NIH in 1969 and has worked on the physiology of the brain ever since. He's stayed one step ahead of the rapidly expanding field by putting his clinical background to good use and by jumping into flow cytometry in its infancy to elucidate and compare phenotypes of individual cells in the brain—critical in the study of development.

Lee Mack

GABA DR Jeffery Barker at work (above) and poised to exit (below).

Lee Mack

The fruition of the last 15 years of effort is what Barker calls the "GABA paradigm of brain development"— a role not previously ascribed to the neurotransmitter. The increased sensitivity of his lab's techniques has revealed that GABA is crucial to spatial development of the brain, as well as to self-regulating feedback loops. Barker feels the work justfies the plate.

"If I should indulge myself in vanity, then let it reflect the scope of the lab,"

Barker says. And thus shall Jeffery Barker be known as **GABA DR**.

Cell Cycles

ASTRCYT and **ANERGY** belong to husband-and-wife scientists **Joan Schwartz and Ron Schwartz**. The Schwartzes got their first vanity plates when they lived in Washington, D.C., where NINDS' Joan Schwartz had "NEURON" and NIAID's Ron Schwartz had "T CELL."

Hers

When they moved to Maryland in 1992, however, Joan Schwartz found that someone already had "NEURON," and she decided to shift the license plate spotlight to her astrocyte work.

Fran Pollner

Joan Schwartz
On an ASTRCYT plane at NIH

Her lab is currently exploring the *moduS operandi* of reactive astrocytes—enlarged cells in the brain formed in response to even subtle injury. Reactive astrocytes may persist long after an injury has healed and have a distinctive pattern of cytokine production and response to neurotransmitters and cytokines—a pattern similar to the signature of immature astrocytes found in neonates.

Working with cultures of normal and reactive rat astrocytes, her team is now focused on identifying factors that cause a normal astrocyte to become reactive and vice versa.

Fran Pollner

Ron Schwartz
Knows ANERGY is where the action is

She says **ASTRCYT** has allowed her to spread a bit of knowlege about science, since she's often asked about her tags by people on the street—who typically figure ASTRCYT has something to do with astronomy. "If the average American knows more about science, it can only be good," Joan Schwartz reckons.

His

Even for the man who has it emblazoned on his license plate, clonal T cell **ANERGY** is still a poorly understood immunological phenomenon—an unresponsiveness that Ron Schwartz and his postdocs induced in some cultured T cells back in 1987.

Cells in this state have essentially turned themselves off—they cease dividing and producing IL-2—but don't go totally quiescent. They can still make gamma interferon, for example.

It took just a few years for the term and the research to catch on among researchers, and it gained additional momentum when another type of nonresponsiveness, this time in B cells, was discovered and dubbed B cell anergy.

By 1990, Ron Schwartz recalls, things started to go crazy. "Anything related to tolerance got called anergy," he says, which led to some backlash against overuse of the term.

As researchers began to understand that normal activation of T cells requires two signals, Ron Schwartz and his coworkers realized that one way in which T cells could go anergic was by receiving only the antigen-specific signal and not the co-stimulatory signal. To date, clonal anergy remains a lab phenomenon, but Ron Schwartz's lab, as well as others, is avidly looking for animal models of anergy and trying to ascertain under what circumstances it would likely play a part in the immune system in vivo.

If ANERGY is poorly understood by immunologists, it's a total enigma to ordinary mortals who glimpse the license plate. Occasionally Ron Schwartz gets an opportunity to educate a few souls about ANERGY, but most folks just assume it has something to do with energy.

Celia Hooper

Lee Mack

ASTRCYT and ANERGY appear to have a predilection for the same parking spot.

Ron Schwartz says ANERGY's inaccessiblity contrasts with his old "T CELL" plates, which were readily understood in this age of AIDS. He recalls one time when he'd parked "T CELL" in Georgetown to go to a movie and returned to find a young man sitting on his car. He yelled at the fellow to get off.

The young man—who turned out to be a medical student—responded defiantly, "Well, what kind of a T CELL are you, anyway, a *suppressor*?" Without a pause and with ostensibly mock gruffness, Ron Schwartz replied, "No, a killer." This got the student off his car and left everyone laughing.

Tribute

Helen Mayberry got her **ONC RN** vanity plate a few years back. She says that most people seem to recognize that RN signifies nurse, but few make the con-

Lee Mack

Helen Mayberry

nection between ONC and oncology or cancer.

Mayberry has been a nurse since 1985, specializing in adult oncology for the last 10 of those years. In 1996, the Fairfax, Virginia, native stepped away from bedside patient care at George Washington University Hospital to join the NIH Clinical Center as a clinical nurse specialist. In this capacity, Mayberry presides over a team of attending nurses, ensuring that they have the skills and knowledge necessary to perform patient care within the letter of various research protocols—a responsibility that underwrites the validity of experimental data.

Although this charge implies a great deal of responsibility for both her and her team, especially in the demanding environs of NIH research, the rewards of working in an adult oncology setting are profound and go beyond the numbers, she says.

"It's a unique opportunity," she reflects, "to see the human spirit rise to the occasion, as patients find ways perhaps they didn't even know about in themselves to deal with their cancer."

Despite the physical and emotional demands of her job, Mayberry feels privileged, she says, to witness the personal growth of individuals who come from very diverse backgrounds to NIH—often for uncertain experimental treatment. ■

Lee Mack

A full plate

Clinical Research Center Moves From the Drawing Boards . . . .

Fran Pollner

Future . . .

In the foreground, NIH director Harold Varmus (left) converses with Sen. Mark Hatfield, for whom the Mark O. Hatfield Clinical Research Center is named. In the background, architect Bob Frasca (left) chats with Clinical Center director John Gallin. Behind and above them all is a drawing of the new CRC, expected to be completed in 2002. A few minutes earlier, as the speech-making inside a very large adjacent tent was coming to an end, Varmus read the plaque that will grace the new facility and that recognizes Senator Hatfield for "his deep and abiding commitment to medical research throughout his years in Congress," where he served in the Senate for 30 years and chaired the appropriations committee for eight before retiring at the end of the last session. Alluding to his own mortality, the senator asked only that the NIH director and everyone else involved in bringing the new CRC to life "please hurry." The assemblage then stepped lively out of the tent to the groundbreaking as the Walt Whitman High School Jazz Ensemble set the pace.

. . . And Past

Among the archival slides NCI's Alan Rabson displayed during his talk at the NIH Research Festival restrospective (see page 9) was this one on the right of the first patient admitted to the Clinical Center. On July 6, 1953, Charles Meredith (seated), a 67-year-old farmer with prostate cancer, passed through the new CC portals to be placed on a hormone therapy protocol by his physician, NCI's Roy Hertz (second from right). Also on hand (left to right) were Clinical Center nurses Nadine Luxmore and Elizabeth Walker, NCI scientific director Bo Mider, and NCI director Rod Heller. Nurses in the fifties, Rabson noted in his talk, were essentially nameless but would not be at the new Clinical Research Center—or anywhere else, thanks to the work of nurses like NINR's director Pat Grady and new scientific director Annette Wysocki (see page 1).

. . . Down to Earth: The Ground Is Broken

Every single day is groundbreaking day at NIH," Vice President Al Gore declared to the assemblage of lawmakers, scientists, and well-wishers gathered within the tent pitched opposite the main entrance of the "original" NIH Clinical Center.

The official groundbreaking ceremony for the Mark O. Hatfield Clinical Research Center took place November 4 on a beautiful, breezy fall morning, ablaze in red and gold leaves.

"The Clinical Research Center will play host to some of the great medical breakthroughs of the 21st century," Gore predicted. His was the first in a series of addresses by political figures, including HHS secretary Donna Shalala, whose efforts on behalf of biomedical research had contributed to the occasion of the day. Shortly after coming to office, the vice president had launched his "reinventing government" agenda and designated NIH's research apparatus a "reinvention laboratory." The new CRC could be considered the centerpiece of that effort.

But not without the money from Congress to do it. Sen. Arlen Specter (R.-Pa.) and Rep. John Porter (R.-Ill.), who vowed to remain partisan to NIH, took their places at the podium, as, of course, did Sen. Mark Hatfield (R.-Oreg.), who urged that greater efforts be made to train clinical researchers, especially in light of the "discouragement" they face these days. Hatfield also underscored the need to enhance support for the NIH-funded General Clinical Research Centers across the country "to translate basic science to the bedside."

Clinical Center Director John Gallin pointed to the core of clinical research at NIH—the patients. Two Clinical Center patients paid tribute to the doctors, nurses, pharmacists, nutritionists—and the science—that have kept them alive and well in the face of their deadly diseases, cystic fibrosis and breast cancer.

Fran Pollner

Digging In: *(left to right) Steve Ficca, director of the Office of Research Services, Tony Clifford, director of the Office of Engineering Services, and Michael Gottesman, deputy director for Intramural Research, put their shoulders to the shovels at the groundbreaking ceremony for the Mark O. Hatfield Clinical Research Center.*

Charles Tolchin, a 29-year-old man with cystic fibrosis diagnosed at age 5 at a time when life expectancy was age 8, listed advances that had allowed him to reach adulthood and to feel strong as he spoke that day, and he thanked NIH for them—the diagnostic sweat test he took at age 5, the anti-inflammatory medications and nebulized antibiotics he needs, the self-operated flutter device he blows into to get rid of secretions, and the double-lung transplant he had last April (at the University of North Carolina) that has eliminated his cough and his need for protracted daily respiratory therapy.

He remarked, too, that NIH had funded the research that isolated the cystic fibrosis gene, "and now NIH is looking for a [gene] delivery system," he added.

Jane Reese-Coulbourne, came to the Clinical Center at the age of 36 after she'd been diagnosed with advanced breast cancer. "I was told I would not likely see 40. I wanted the most aggressive therapy I could find, and I came to NIH on protocol. 'Your cancer appears to be gone'—they've been telling me that now for the last seven years," she recounted.

"We need more patients in clinical trials on experimental therapies," she concluded. ■

—Fran Pollner

Cell Processing Facility Debuts

Janet Yee

On July 1, 1997, Harvey Klein (left), the Clinical Center's transfusion medicine chief, ushered in the new 3,000-square-foot cell-processing facility, created to isolate cellular components needed for intramural clinical trials, including hematopoietic progenitor cells, mononuclear cells, and other subsets of lymphocytes typically used in immunotherapy, gene therapy, and stem cell transplantion research. The facility is funded by the Clinical Center and Baxter Healthcare through a CRADA. Potential users of the service should contact Elizabeth Read (right), chief of the cell processing section.

Catalytic Reactions

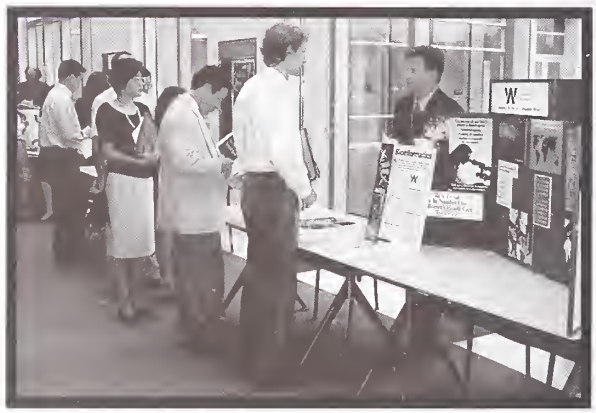
Below are comments we received in response to questions posed or issues raised in recent issues.

On collaboration quandaries

In my opinion, there are four major problems with collaborations. First is the requirement that all authors be fully responsible for all data included in a publication. A tremendous amount of trust is required, especially with a long distance collaboration or a collaboration between persons in very different fields. The second is that genrally first or senior authorships carry the most weight in hiring, promotion, or tenure decisions since, on average, only one-half of collaborative papers will result in a senior or first authorship. The third is that for a tenure-track person, collaboration usually means working with a more established lab. Even if a tenure-track person is senior author on a paper with a better known researcher, the paper may often be associated with the "bigger" name in oral presentations by others in the field. The fourth is that letters of recommendation for promotion and tenure from collaborators usually carry less weight. One often has to balance the value of collaborating with a giant in one's field against losing that person as a recommender.

—anonymous

NIH Research Festival: All Out For New Data and New Jobs



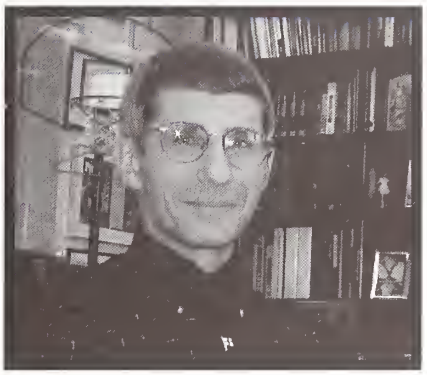
Postdoc Line-Ups. *For three hours one Festival morning, 20 prospective industrial and governmental employers set up shop at Natcher to lure the prestigious, proud, personable, and practically penniless postdoc population from the NIH labs they must sooner or later exit to theoretically greener worksites. (A name, address, and contact for each of these can be found at <ftp://helix.nih.gov/felcom/index.html>.) The Job Fair's only competition was a three-hour workshop, pictured below.*

Sit-Ins. *While their colleagues queued up to shake hands and exchange information with potential employers, these stalwart scientists nested into a workshop one floor below, where NIDA and NIMH investigators presented their findings on how "Transgenic Mice Provide New Evidence about Drug Actions in the Brain." Here, NIMH's Christine Wichems elaborates on the information gleaned from serotonin-transporter knockout mice on the actions of psychostimulant drugs.*

Photos by Fran Pollner

A Festival Retrospective: Celebrating 60 Years of Bethesda-Based Intramural Research

HIV Found in Resting Blood Cells Of Patients with 'Virus-Free' Plasma



Fran Pollner

Anthony Fauci

Patients on highly active triple-combination therapy whose plasma HIV levels had diminished below the vanishing point of commercial detection assays still had detectable virus in their peripheral blood cells, according to a Clinical Center study involving 18 HIV+ patients.

"Virus was not only detectable (in the resting CD4-positive T cells) but culturable and replication-competent," NIAID director Anthony Fauci told attendees at an NIH Research Festival symposium at which of-the-moment reports were not expected among the scheduled array of 60-year reminiscences. [He'd presented these findings a few weeks earlier at the Gallo lab meeting, a yearly AIDS research gathering hosted by Robert Gallo, formerly of NCI and now at his Institute of Human Virology in Baltimore, and they were to be aired again in October at an AIDS meeting outside Paris, Fauci later said.]

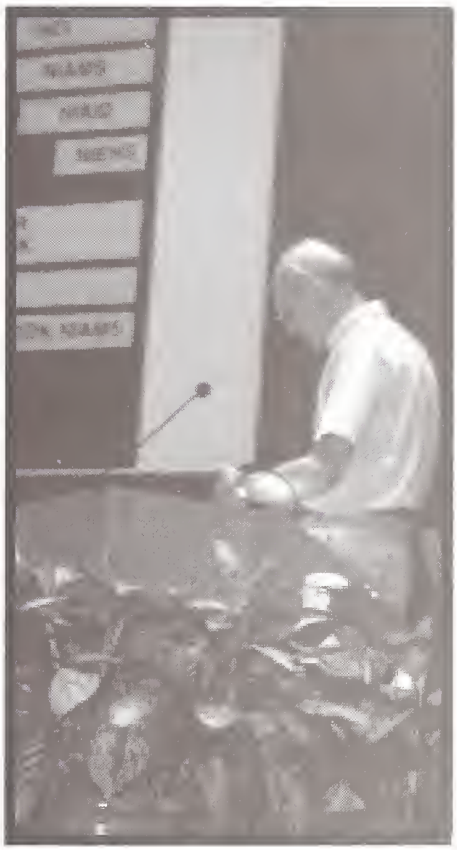
The 18 patients included five whose blood was sampled before treatment began and 13 who'd been on potent antiretroviral combination therapy for up to 10 months. Nine of these 13 had no detectable virus in plasma.

The frequency of integrated HIV DNA "was about the same in all three groups. . . . not very good news," Fauci observed. The cells also carried unintegrated HIV DNA, he said, noting that "if the virus were truly suppressed, we would have expected the unintegrated form to have disappeared." The presence of unintegrated HIV DNA within the cell "suggests—doesn't prove, but strongly suggests—that that cell was recently infected. If all you saw was integrated virus, you really wouldn't know how long that virus had been there. It could have been for years," Fauci later said. He noted that at least two other groups—Doug Richman's at UCSD and Bob Siliciano's at Johns Hopkins—have been doing similar studies and getting similar results. A Siliciano co-investigator is David Ho, who led a team that early last year reported that triple therapy that included a protease inhibitor was yielding undetectable plasma virus levels.

The new findings, Fauci emphasized, "do not at all indict the drugs we have, which are quite good. Just because we haven't been able to completely eliminate the virus does not mean we should not continue to try. Suppressing plasma viremia has yielded striking results with very good clinical benefit." What's needed, he said, are even more potent drugs, against the same targets and against new ones like integrase, the enzyme that enables HIV DNA to integrate into the cell's genes—as well as "creative ways to rid the body of this reservoir of resting, latently infected cells." He suggested also that knowing the half-life of resting CD4 cells could lead to better projections of "how long before we can discontinue therapy with a reasonable degree of comfort." ■

—Fran Pollner

Among NIH scientists looking back over the 60-year intramural research record were Alan Rabson, NCI deputy director, who recalled that back in the fifties "oncologists were the poison doctors of clinical medicine" and then paid tribute to such NCI luminaries as Roy Hertz, Bo Mider, Vince DeVita, Robert Young, Marc Lipmann, and Steve Rosenberg.



David Davies, chief of the organic chemistry section at the NIDDK molecular biology lab, tracked the insinuation of structural biology into the central nervous system of every institute on campus (in North Carolina as well as Maryland). As crystallography became an integral part of NIH labs, protein models shrunk from the giant Lego-like structures housed in the DeWitt Stetten, Jr., Museum of Medical Research to the computer images that appear and vanish at the click of a mouse.

NIH Director Harold Varmus looked ahead to the completion of the new Clinical Research Center and its research and training links with centers across the country. The IRP, he said, will retain its agility in responding to "special needs," reflected now in the marshalling of vaccine forces to design an AIDS vaccine—and, later, vaccines against such diseases as TB and malaria.

The Science of Nursing
continued from page 1

rector, a nurse-scientist who would shape and strengthen the NINR intramural research program and commit herself as well to training a new generation of nurse scientists.

Wysocki, formerly director of nursing research at New York University and an NINR-funded investigator, answered the call and arrived in mid-September this year. Although the end of the fiscal year is not an auspicious time to secure supplies, she began—with gusto—to set up her lab, "the first Nursing Institute lab ever on the NIH campus," she remarks. But not the last. Both she and Grady see a major expansion in the next few years in the NINR intramural research program. The 2% of the NINR budget that intramural research currently commands will surely expand, Grady asserts, projecting the initiation of five or six research protocols—clinical and basic—over the next couple of years and the recruitment of more intramural scientists.

"We're interested in bringing young extramural people here as postdocs—and people in mid-career who want to learn new things," Grady says. "A critical issue in this field," she adds, "with so many young people wanting training, is that our mentors are overworked. What we're trying to do is get each mentor to train their trainees to be mentors."

NINR has also pioneered a summer course to teach nurses how to do clinical research and how to secure a research grant. Four hundred applied for 25 slots the first time around two summers ago, Grady recounts. This past summer, there were 50 slots available.

Another of Grady's goals is to recreate at the intramural level the strong research collaborations NINR has developed extramurally with the other NIH institutes. Grady was a member of the Straus panel and Wysocki is on the implementation committee formed to carry out the recommendations (see the September-October issue of *The NIH Catalyst*, p. 1); Grady is also on the Board of Governors of the new Clinical Research Center and, she says, Wysocki "is on the frontlines of the space issues" related to the new facility.

Wysocki is talking to other scientific directors about creating a training program for nurses who want to pursue research in areas that fall within their purview. "We want to sponsor IRTAs in labs and branches across the campus. In the immediate future, I'd like to recruit up to four IRTAs—ideally for three years and certainly no less than two years—and then expand that number. There needs to be a more formal pathway created for scientists who are nurses and are interested in doing bench-based research, and that's one of the reasons I took this position," Wysocki says.

Fran Pollner

Annette Wysocki
"There needs to be a more formal pathway created for scientists who are nurses and are interested in doing bench-based research."

In her own research, Wysocki is on the front-lines of wound healing. Following are excerpts from an interview last month with the new NINR scientific director.

Q: What ignited your interest in research?
Wysocki: I was a staff nurse at the University of Virginia Medical Center in Charlottesville, and there happened to be clinical research studies on pain being conducted on our unit. That peaked my research interest and kept it alive. But I also knew that I needed to obtain an advanced degree to become a principal investigator; a baccalaureate was not going to be enough.

Q: Because you wanted to do clinical research?
Wysocki: Because I wanted to do research with implications for patients.

Q: What's the pathway to basic research when your initial preparation is in nursing?
Wysocki: There are courses and programs in cell and molecular biology that nurses who want to become trained in basic science research can enroll in, just as any other medical or undergraduate student would. And they can engage in research training opportunities here at NINR/NIH or at their own university.

Q: What would distinguish a nurse's approach to cell and molecular biology from a physician's? What's the difference between an M.D./Ph.D. and an R.N/Ph.D?
Wysocki: The boundaries here are very fuzzy. But the problems that nurses identify and choose to study arise from working directly with patients on a day-to-day, 24-hour-a-day basis. We're on the front-lines delivering care directed at the relief of pain and treatment of wounds. We are the only practitioner that provides continuous monitoring and observation of patients, minute to minute, hour to hour. This creates powerful clinical observations that drive the research questions that nurses formulate—basic research questions and other types of questions. We have an intimate knowledge of what's going on with the patient—biologically, physically, emotionally, ethically, and with their families.

I've been studying wounds since 1984, before my postdoc. My doctoral work involved a wound healing study, but in the course of doing that study, I realized I needed cell and molecular biology, so I did a postdoc in the Depart-

ment of Cell Biology and Anatomy at the University of Texas-Southwestern Medical Center in Dallas and at Cornell University Medical College in the Surgery Department [both as an NIH postdoctoral research fellow].

Because of my clinical background, I concentrated on the clinical questions. That's what was driving my science. But now my science is driving my clinical practice to some extent, because we're starting to find some interesting things that may have clinical implications.

Q: Is your coming to NIH a reflection of a new approach for the Nursing Institute?
Wysocki: Definitely. My coming here reflects a greater emphasis on a research program not only with a clinical component but a solid laboratory component to stand behind the clinical questions. We want to build on the clinical studies that were carried out previously at the Clinical Center and create a solid training pathway for future research scientists.

Q: What are some of those clinical questions?
Wysocki: Pain is still a hot topic for us, and it will continue to be as Americans grow older and suffer from chronic conditions, especially from the vantage of home care, since patients are discharged earlier to their homes in the care of nurses.

There's a whole host of problems and opportunities: immobility stress, relaxation, sleep, loss of appetite, nausea, dementia, depression, and recovery from stroke. And, of course, chronic wound care is still a big problem in this country, costing approximately $9 billion a year.

I'm interested in chronic nonhealing or slow-healing wounds, in the clinical context of leg ulcers, which arise for a variety of reasons, and pressure sores—especially related to spinal cord injury, which I'd like to work on with the Paralyzed Veterans of America.

Q: How will you pursue that?
Wysocki: We'll be setting up clinical protocols to recruit patients with leg ulcers. In addition to the clinical workup, I collect wound fluid and tissue samples and bring these materials into my lab to be analyzed to see what it is that's not occurring in these wounds that does occur in wounds that heal normally—after surgery or following a relatively minor scrape. At the moment, I'm interested in looking at proteases, specifically matrix metalloproteases. My work has shown that there's an overexpression of matrix metalloproteases in chronic wounds—not all of them, but some of them.

And what I see in the lab makes me see the wounds clinically all over again. It's exciting. It makes them new again.

Q: Can you look at a patient clinically and estimate what the odds are of normal wound healing? Can you anticipate difficulty based on the kind of patient you see or what the wound looks like?
Wysocki: I couldn't do that confidently yet. I've always wanted to do an epidemiological study of wounds, to look at other risk factors. Looking at you today, I can't predict if you'll have problems with leg ulcers as you get older—unless you have diabetes or a circulatory impairment.

Q: Would you describe your research as basic, clinical, or a combination?
Wysocki: It's a hybrid. I'm dancing on both sides of the aisle. I think basic science takes a reductionist approach, which is useful to look at mechanisms, give you a basic understanding. But at some point, in this multifactorial world, you have to be an integrationist. There are going to be predominating factors, and you have to find out which ones are winning on an in vivo level, on a patient level.

Anyone who's doing science that has relevance for human health has to be both a reductionist and an integrationist to have the greatest impact. ■

Fran Pollner

Pat Grady

"With so many young people wanting training, our mentors are overworked. What we're trying to do is get each mentor to train their trainees to be mentors."

Research Fellowships Beckon from Japan

Through arrangements made with the Fogarty International Center, The Japan Society for the Promotion of Science (JSPS) is offering fellowships for American biomedical and behavioral researchers to pursue collaborative research in Japanese universities and other eligible institutions and laboratories.

The JSPS Short-term Fellowship provides for stays of 7 to 60 days; the JSPS Short-term Postdoctoral Fellowship runs from 3 to 11 months. Applicants must be U.S. citizens or permanent residents and research plans must be arranged in advance with the Japanese host. The application deadline is January 30, 1998.

Interested persons should contact the FIC immediately to receive detailed program information and application instructions. Requests should be addressed to: Allen Holt or Christina McLauchlan at 496-4784; fax:480-3414; e-mail: <jsps@nih.gov>. ■

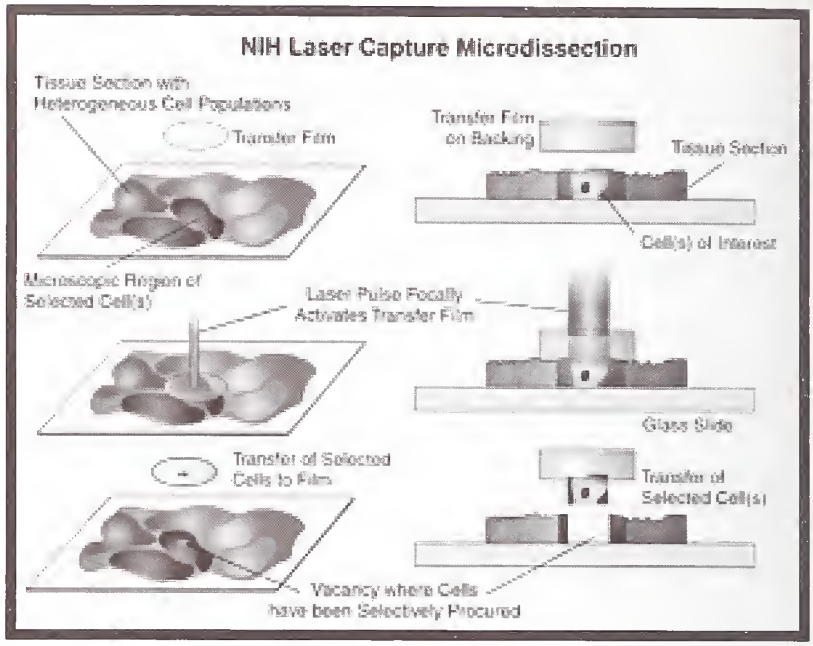
Laser Capture Microdissection Facility Brings Molecular Pathology To NIH Community

Pathologists have long been able to correlate histological changes with the progression of cancer. But that is no longer enough. As the Human Genome Project and the Cancer Genome Anatomy Project identify all expressed human genes, medicine will be faced with its next challenge: elucidating the function of the genes and characterizing their interaction. For pathology, this will mean being able to correlate morphological changes with specific genetic events (1).

Over the past decade, the polymerase chain reaction (PCR) has allowed the robust analysis of very small amounts of tissue; nevertheless, a major obstacle in applying PCR to tissue is the inherent heterogeneity of the cell populations. In a gross biopsy specimen from a cancer patient, for example, the neoplasia may only constitute a small fraction of the total tissue mass. Inflammatory cells, stromal elements such as fibroblasts, and normal tissue will inevitably be present and will mar the molecular analysis of the tumors. The tumors themselves may be polyclonal in origin, further complicating genetic analysis. Finally, haphazard sampling of the cells in the tissue may disturb the normal gene expression pattern regulated by cell-cell and cell-matrix interactions and other local environmental factors.

To address these issues, microdissection techniques have been developed, and these have moved from comparatively tedious and difficult manual maneuvers to the more elegant approach used by Shibata (2). In this technique, a protective dye is applied to the areas of interest and ultraviolet radiation is used to ablate the unprotected tissue. The tissue areas of interest remain untreated and can be scraped manually and collected for study. A more automated technique involves placing the tissue section on a film and using an ultraviolet laser to ablate the unwanted regions, leaving islands of exposed regions of interest (3). The selected islands are then procured with a needle. But even this method becomes tedious when small foci are the targets of dissection—the desired areas must still be circumscribed by the ablation and collected one by one.

The newest generation of automated microdissection is laser capture microdissection (LCM) (4), developed via the intramural collaborative efforts of NCI and BEIP. The technology is being commercialized through a Cooperative Research and Development Agreement with Arcturus Engineering, Inc.



Unlike previous microdissection methods, LCM operates by positive rather than negative selection. First, a clear-transfer film is applied to the surface of the tissue. An infrared laser melts the film in very focused areas, targeting for capture only the cell(s) of interest. The rest of the tissue section is left behind. No tissue is destroyed in the process. Direct visualization of the transferred tissue, with its histology intact, is then possible.

The commercially available LCM system, called PixCell, offers additional advantages: The transfer film is on the underside of a cap that fits into a standard 500µl Eppendorf tube, which facilitates digestion of the tissue and procurement of a PCR template. Also, a soon-to-be automated process of loading and unloading the caps will greatly reduce the possibility of contamination, an important consideration for PCR.

NCI is making its core LCM facility available to all NIH investigators. Several LCM microscopes will be available, with technical assistance and pathologists standing by. Interested investigators can call and set up an appointment to bring their material. If the starting material is properly prepared, the microdissection session can be quite short, and the investigator can leave with the microdissected cells ready for analysis. LCM training conferences are being held every two months. To register for the course, contact Robert Bonner at 301-435-1946. You must attend the LCM training conference before using the Center.

Tissue Preparation

Embedding

Typically, tissues must be fixed, dehydrated, cleared, embedded, sectioned, and mounted. Each of these steps can influence the efficiency of the LCM transfer and the subsequent PCR analysis. We have found the following protocol works best for LCM of paraffin-embedded tissues (PET).

1. Fix tissue in 10% neutral buffered formalin (NBF) or 70% ethanol for 2.5–4.0 hours for 3-mm-thick tissue slices.

Fixation is performed to preserve the morphology of the living tissue, but it does not necessarily have a beneficial effect on the DNA. Formalin, one of the most popular fixatives, cross-links DNA to protein, thus making the DNA molecule rigid and susceptible to mechanical shearing during the handling of DNA-containing aqueous solutions (5). Direct cross-linking of DNA strands or of DNA to protein will also prevent polymerase reading through these sites and reduce the effective DNA length for PCR. The DNA yield from formalin-fixed tissues decreases with prolonged fixation time, but the loss is acceptable if routinely processed specimens are fixed for less than 24 hours. DNA yield and quality are improved following ethanol fixation and appear unaffected by the duration of fixation (6). We are now developing ethanol-based fixation procedures.

2. After fixation, the following steps are

Disclaimer: *Mention of specific products in this article does not constitute an endorsement of those products, nor does it signify that other similar products are less desirable.*

by Chetan Seshandri, Mary Huckabee, Nicole Simone, Michael Emmert-Buck, and Lance Liotta, Laboratory of Pathology, DBS, NCI, and Robert Bonner, Laboratory of Integrative and Medical Biophysics, NICHD

performed in an automated tissue-processing machine.

Processing can be completed routinely overnight, or it may be handled on an accelerated basis. We have found no change in the LCM transfer efficiency for tissues processed via the accelerated cycle.

Routine Overnight Processing

Station	Solution	Conc.	Time	Temp. (°C)
1	NBF	10%	2:00	40
2	NBF	10%	2:00	40
3	Ethanol	70%	0:30	40
4	Ethanol	80%	0:30	40
5	Ethanol	95%	0:45	40
6	Ethanol	95%	0:45	40
7	Ethanol	100%	0:45	40
8	Ethanol	100%	0:45	40
9	Xylene	100%	0:45	40
10	Xylene	100%	0:45	40
11	VIP Paraffin		0:30	58
12	VIP Paraffin		0:30	58
13	VIP Paraffin		0:30	58
14	VIP Paraffin		0:30	58

Accelerated Processing

Station	Solution	Conc.	Time	Temp. (°C)
1	Ethanol	70%	0:10	40
2	Ethanol	80%	0:10	40
3	Ethanol	95%	0:15	40
4	Ethanol	100%	0:20	40
5	Ethanol	100%	0:30	40
6	Xylene	100%	0:30	40
7	Xylene	100%	0:30	40
8	Xylene	100%	0:30	40
11	Paraffin		0:20	60
12	Paraffin		0:20	60
13	Paraffin		0:30	60
14	Paraffin		0:20	60

3. Block the wax and specimen out in a mold.

4. Cut sections on a clean microtome with a clean blade.

5. Float paraffin ribbons on 43-44°C deionized water (NO ADHESIVES).

6. Mount on plain, uncoated glass slides.

7. Dry slides in a 37°C oven for one hour.

Paraffin embedding can be used for samples destined for DNA analysis, but it causes damage to RNA; thus, frozen tissue should be used for RNA analysis. Embedding and sectioning frozen tissue, as described by Kiernan (7), yields slides suitable for LCM .

Staining

Staining should be performed as indicated below, using solution baths that are replaced regularly. Note that the times for hematoxylin and eosin staining are dramatically shorter than normal histology would require. We believe longer staining will reduce the efficiency of transfer and decrease the length of DNA fragments that can be recovered. For DNA analysis of a paraffin-embedded section, begin staining procedures with deparaffinizing (step #1 below). For a frozen embedded section for RNA analysis, begin with step #6, using 70% ethanol to quickly fix the section.

1. Soak slides for 5 min in xylene to deparaffinize them.

2. Rinse for an additional 5 min in clean xylene.

3. Dip slides 12 times (approx. 5 sec each dip) in 100% ethanol.

4. Dip for 5 seconds in a clean bath of 100% ethanol.

5. Dip slides 12 times (approx. 5 sec each dip) in 95% ethanol.

6. Dip slides 12 times (approx. 5 sec each dip) in 70% ethanol.

7. Dip slides 12 times (approx. 5 sec each dip) in purified water.

8. Dip the slides for 10-15 sec in Mayer's hematoxylin.

9. Dip slides 12 times (approx. 5 sec each dip) in purified water.

10. Submerse slides for 10-15 sec in bluing reagent.

11. Dip slides 12 times (approx. 5 sec each dip) in 70% ethanol.

12. Dip slides 12 times in 95% ethanol

13. Submerse slides in Eosin Y for 30-60 sec.

14. Dip slides 12 times (approx. 5 sec each dip) in 95% ethanol.

15. Dip slides 12 times in a clean bath of 95% ethanol.

16. Dip slides 12 times in 100% ethanol.

17. Submerse slides for 30-60 sec in xylene.

18. Shake off excess xylene and wipe slides carefully with particle-free paper towel or tissue

19. Air dry slides at least 2 min to allow xylene to evaporate completely.

Microdissection (LCM)

Slides with fixed, sectioned, stained tissue should be carefully labelled, including information on sample thickness, staining, and coating of slide, as well as identification of the tissue targeted for transfer (e.g., invasive cancer, normal epithelium, lymphocytes), fixation protocol, and the actual section number to be captured if it is a serial section.

The sample thickness will be useful in automatic computation of the volume of transfer from spot size, number of laser spots, and the percent transferred within a spot. Prepared slides are then brought to the LCM Center, located in Building 10, Room 2C-401.

To sign up for a user slot, call Bob Bonner at 435-1946, Lance Liotta at 496-2035, or Michael Emmert-Buck at 496-2912. Collaborators may set up as much as a three-hour time block to work with a Laser Lab staff member. These times are either 10 a.m.–1 p.m. or 2 p.m.–5 p.m. daily.

At the Center, a computer program created by DCRT will guide users in turning on the microscope, microscope monitor, vacuum pump, and the laser, and will establish a file folder for each user's study.

The computer will prompt the user to enter data on the slide number and the specific tissue targeted for transfer. Careful recording of this informatin will allow correlation of LCM images relative to the coverslipped slide, permitting use of adjacent sections for high quality roadmap images.

After entering additional cap and identification information for tracking the job, the user can add additional optional information. Next, the program prompts input of the laser parameters, including spot size, pulse power, pulse duration, and sample thickness. These critical values are used by the computer to control the laser. Presently, spot size is set by the user.

A 60-µm beam is selected by using a weight with a defocusing lens, and a 30-µm beam is obtained by using a weight without the lens. This value is important to include for reference.

We recommend setting the pulse power to 30 mW, the pulse duration to 50 ms, and the beam size to 60 µm. A 60-µm beam should be used for homogeneous tissue for more rapid transfers. When the targeted cell groups within the

tissue are smaller than 60 µm, then the 30-µm beam will be used.

After valid entries have been recorded for user, study, slide number, cap information, laser pulse duration, and pulse power, LCM will proceed, with all this information recorded in the data record file of the transfer, along with any images taken.

The user can acquire images and begin LCM transfer. The estimated percent transferred can be adjusted after the dissection if the user feels that the default 90% value is incorrect.

Next, the user selects a coverslipped slide and obtains roadmap images. If the user has serial sections, it is best to coverslip the middle slide. The computer allows users to select, annotate, display, and review roadmap images, with options for voice annotation and web capture of these images.

For all subsequent procedures, users should wear gloves to avoid contaminating the microtransfers or leaving finger oils on extraction vessel tubes. Finger oils make it difficult to write on the tubes.

During the transfer process, users center the viewing area with a joystick and then rotate the microscope turret to center the 4X objective over the stage. At this magnification, the view in the eyepiece is almost exactly the size of the film surface on the cap. This is the region within which transfers can be made on a single cap. Users position the slide to cover vacuum holes and then use the cap-loading device to load a cap onto the cap rack, taking care to avoid any contact with the film surface. Multiple caps can be loaded and used successively.

Next, users operate the cap-manipulating arm to position and drop the cap onto the tissue. The position of the laser spot is indicated by the low-intensity aiming beam on the computer screen. Tissue can be selected and transferred at 4X, 10X, 20X, or 40X magnification.

Users next fire a series of laser pulses, moving the stage between pulses to select different target clusters. Before the tissue is removed, users may take and annotate "before dissection" pictures. They then quickly remove the cap. Time is of the essence here to prevent the polymer from deforming and contracting without removing all the tissue.

Users check the original tissue sample to verify the transfer has occurred and may take and annotate "after dissection" pictures before positioning a clean glass slide on the center of the microscope stage. Users may also photograph the tissue that has been removed by snapping a "cap image."

Before concluding, users review their images, edit any parameters that have been altered, write all the data to their file, label the Eppendorf tube that will hold the sample (date, author, study, case number, slide number, tissue type, number of cells, etc.), verify that it contains sufficient digestion buffer (e.g., 50 µl buffer aliquoted from solution containing 20 µl 1% proteinase K, 480 µl 10mM Tris-HCl [pH=8.0], 1mM EDTA, 1% Tween-20), cap the tube, and turn it upside down, shaking gently until the buffer drops to the cap surface.

Molecular Analysis

Users then return to their labs with the LCM samples and conduct molecular analyses. Thanks to the greater homogeneity of populations of cells extracted via LCM, molecular analysis is significantly enhanced and can be extended to more applications. DNA and RNA extraction and amplification has become more efficient; cDNA libraries (8) and microarrays (9) can be used to discover the functions and interactions of genes.

For DNA extraction, procedures used by Emmert-Buck (10,11) are recommended. Buffered samples from the LCM are incubated overnight at 37° C and are centrifuged for 5 min. Next, the tube caps are removed and the samples boiled for 8 min at 95° C to inactivate proteinase K. The samples can then be used as templates for PCR.

For RNA isolation, procedures modified from the Stratagene Micro Isolation Kit, Catalog #200344, may be used and scaled down for size and purity according to the protocol in Schena et al. (9).

Troubleshooting Tips

Tissue Prep

1. Guard against excessive adhesion of tissue to the slide. For a successful LCM transfer, the strength of the bond between polymer film and the targeted tissue must be stronger than that between the tissue and the underlying glass slide. We observe reduced efficiency of transfer with slides that have been charge-treated (poly-L-lysine) to increase tissue adherence to the slide. Baking the sample onto the slide may denature the interface surface and bond it too strongly. Some histopathology labs use an adhesive in the water bath to improve the tissue section adhesion to the slide, and this may result in reduced or variable LCM transfer. However, when we use plain glass slides that are not charged or coated, we achieve consistent (100 out of 100 30-60µm diameter spots) LCM transfers from 4-10µm thick PET sections.

2. Mount the tissue as close to the center of the slide as possible; otherwise it may be difficult to lock the slide down to the stage and dissect the area of interest.

3. The microtome used to cut sections should be kept clean: excess paraffin and tissue fragments should be wiped away with xylene and a fresh microtome blade should be used for each block.

4. Store prepared tissue slides at a moderate temperature (18° C) and humidity (45%) until required for LCM transfer. Stain tissue just before LCM transfer time.

Staining

1. Filter hematoxylin and bluing solution to remove precipitates; do not allow 100% ethanol to hydrate; be sure to completely deparaffinize slides with xylene. After staining, a final xylene rinse is most critical for tissue transfer. Whenever transfer is inadequate, you can repeat staining procedure steps 11, 12, 14-19.

2. Sections must be dehydrated and not coverslipped for effective LCM transfer. This makes the staining appear darker and more granular. Where the polymer melts and bonds to the targeted tissue, it will appear lighter. A diffuser film on the slides, which we can supply, will dramatically improve imaging.

Transfer

If nonspecific transfer is noted in the "CAP IMAGE," it can be removed. A methanol wash of the cap surface will eliminate loose dirt and tissue. For material that is more firmly held, apply a piece of Scotch tape to the cap surface and peel it away quickly. The removed debris can be visualized by placing the piece of tape on a clean glass slide.

Extraction and Amplification

1. The cap should be inserted into the standard Eppendorf 0.5-ml tube with a special tool to ensure there is a 0.0625-in gap between the top of the cap and the tube rim..

2. There is a considerable range in the nucleotide sequence length that can be expected to produce good PCR results. Some investigators estimate the limit on amplification products from PET sections to be as low as 80-170 base pairs (12). However, most of the current literature (12–15) and our own experience suggest that products around and below 400bp can be expected. We have succeeded in amplifying a 220-bp product from a cap with as few as ten 60µm transfers containing 100 cells. The template concen-

tration was 2 cells/µl.

3. For fragments less than 250 bp, or analysis requiring resolution of fragments of similar size, separation of the PCR products by polyacrylamide gel electrophoresis (PAGE) is recommended. With the small amounts of tissue garnered from microdissection, PCR products can be labeled with ^{32}P for increased sensitivity (10,11).

Concluding Remarks

The protocol presented here has been verified by a user group trial and represents a guideline for the present use of the technology. But the technique is evolving rapidly. Modified protocols are being developed for the use of alternative staining techniques, including immunohistochemistry.

Better imaging during transfer, for example through an index-matching fluid, is also being explored. Although dissection is recommended at 30 or 60µm for reproducibility, preliminary results indicate that transfers as small as 10µm may soon become standard.

LCM will, for the first time, allow researchers to study any disease process at the molecular level. In oncology, this will lead to an understanding of the progression of cancer through various premalignant stages, all of which can be dissected and analyzed. In infectious diseases, it will lead to a more precise understanding of infection, as well as the effects of treatment, at the cellular level. In developmental anatomy, we can begin to catalog the myriad focal changes that have far-reaching effects. We expect that as the technology itself evolves, laser capture microdissection will continue to redefine the state of the art in these fields. ■

Resource People

RNA recovery: Kristina Cole, NCI, 6-3379
DNA recovery: Jeff Lee, NCI, 6-2912
Human tumor LOH: Michael Birrer, NCI, birrerm@bprb.nci.nih.gov
Human pathology: Rodrigo Chuaqui, NCI, 6-3379
cDNA libraries: David Krizman, NCI, 5-5155
LCM commercialization: Arcturus Engineering, Inc. (408 654-7937)

The LCM web site, which includes a description of the core lab, procedures, instruments, and protocols is at **<http://dir.nichd.nih.gov/lcm/lcm.htm>**

References

1. R.F. Bonner, M. Emmert-Buck, K. Cole, T. Pohida, R. Chuaqui, S. Goldstein, and L.A. Liotta. "Laser capture microdissection: molecular analysis of tissue." *Science* **278:**1481–1483 (1997).

2. D. Shibata, D. Hawes, Z. Li, et al. "Specific genetic analysis of microscopic tissue after selective ultraviolet radiation fractionation and the polymerase chain reaction." *Am. J. Pathol.* **141**:539–543 (1992).

3. M. Bohm, I. Weiland, K. Schutze, et al. "Microbeam MOMeNT: noncontact laser microdissection of membrane-mounted native tissue." *Am. J. Pathol.*. (in press).

4. M.R. Emmert-Buck, R.F. Bonner, et al. "Laser capture microdissection." *Science* **274**:998–1001 (1996).

5. C. Mies. "Molecular biological analysis of paraffin-embedded tissues." *Human Path.* **25**:555–560 (1994).

6. D. Crisan and J.C. Mattson. "Retrospective DNA analysis using fixed tissue specimens." *DNA and Cell Bio.* **12**:455464 (1993).

7. J.A. Kiernan. *Histological and Histochemical Methods: Theory and Practice.* Second Edition. Pergamon Press, New York. (1990).

8. D.B. Krizman, R.F. Chuaqui, P.S. Mettler, J. M. Trent, P.H. Duray, W.M. Linehan, L.A. Liotta, and M.R. Emmert-Buck. "Construction of a representative cDNA library from prostatic intraepithelial neoplasia. *Cancer Research* **56**:5380–5383 (1996).

9. M. Schena, D. Shalon, R.W. Davis, and P.O. Brown. "Quantitative monitoring of gene expression patterns with a complementary DNA microarray." *Science* **270:**467–470 (1995).

10. M.R. Emmert-Buck, C.D. Vocke, R.O. Pozzati, P.H. Duray, S.B. Jennings, C.D. Florence, Z. Zhuang, D.G. Bostwick, L.A. Liotta, and W.M. Linehan. "Allelic loss on chromosome 8p12-21 in microdissected prostatic intraepithelial neoplasia." *Cancer Research* **55**:2959–2962 (1995).

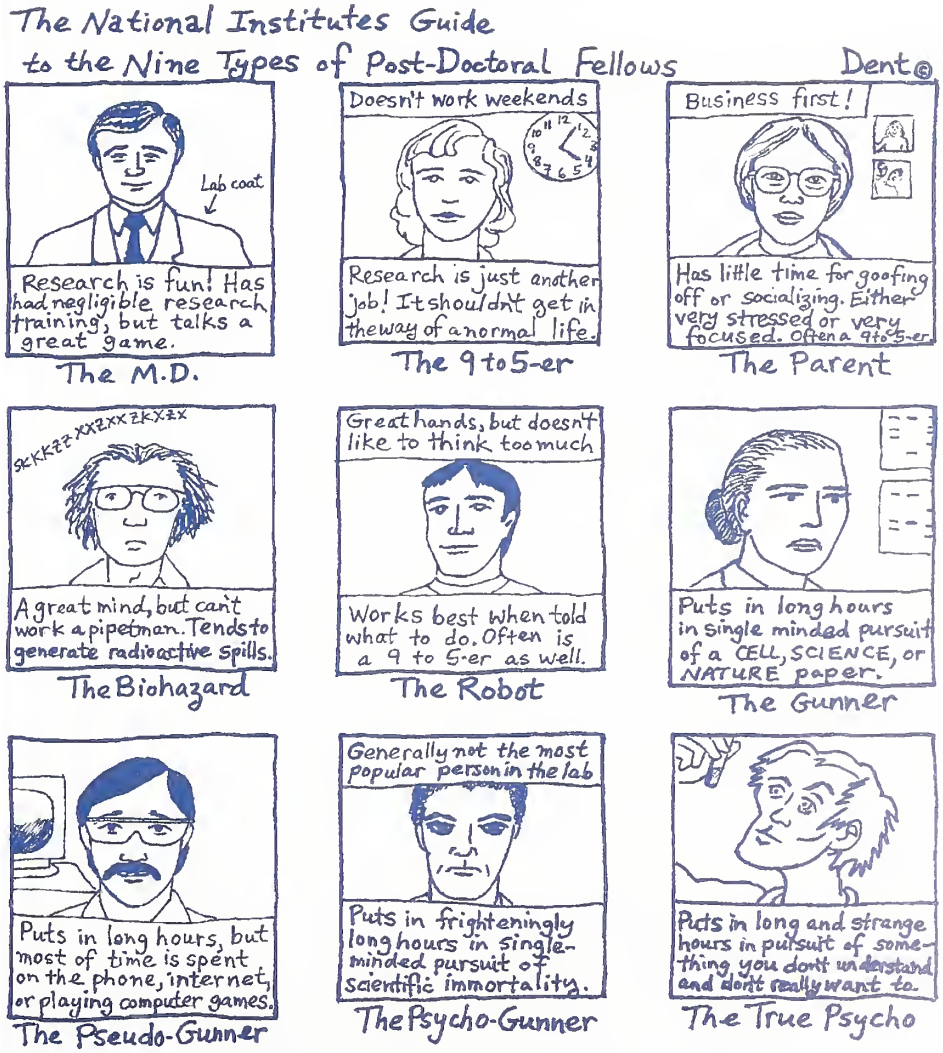
11. Z. Zhuang, M.J. Merino, R. Chuaqui, L.A. Liotta, and M.R. Emmert-Buck. "Identical allelic loss on chromosome 11q13 in microdissected in situ and invasive human breast cancer." *Cancer Research* **55:**467–471 (1995).

12. D. Shibata. "Extraction of DNA from paraffin-embedded tissue for analysis by polymerase chain reaction: new tricks from an old friend." *Human Path.* **25:**561-563 (1994).

13. L.X. Pan, D.C. Diss, and P.G Isaacson. "The polymerase chain reaction in histopathology." *Histopath.* **26:**201–217 (1995).

14. M. Loda. "Polymerase chain reaction-based methods for the detection of mutations in oncogenes and tumor suppressor genes." *Human Path.* **26:**564-570 (1994).

15. L. Whetsell, G. Maw, N. Nandon, D.P. Ringer, and F.V. Schaefer. "Polymerase chain reaction mmicroanalysis of tumors from stained histological slides." *Oncogene* **7:**2355–2360 (1992).



Call for Catalytic Reactions

In this issue, we are asking for your reactions in four areas: great expectations for postdocs and the new Clinical Research Center, "hot methods," and two- versus three-dimensional reading material. **Send your responses on these topics or your comments on other intramural research concerns to us via e-mail: <catalyst@nih.gov>; fax:402-4303; or mail: Building 1, Room 209.**

In Future Issues...

- Pre-IRTA Permutations
- The Arthur Anderson Opus
- A Bombing Mission For Triplex DNA

1) Is the list of ten expectations of a good postdoctoral experience reasonable? What would you add, delete, change?

2) What are your expectations of the new Clinical Research Center? How can NIH best use the new facility to revitalize clinical research?

3) Laser Capture Microdissection may be a tough act to follow, but we know there are other "hot methods" out there. Suggestions?

4) Hard copies—does NIH need them? *The NIH Catalyst* and the NIH Yellow Sheet (Calendar of Events) are two documents available both in hard copy and on-line. Should we retain both electronic and print versions?

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